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SCREENING ASSAY

Technical field

- 5 The present invention relates to a method of identifying compounds which are capable of acting as agonists or antagonists of G-protein coupled receptors (GPCRs). Such compounds may be used as, or further developed into, drugs for activation or deactivation of GPCRs. The present method is advantageously used as a high throughput screening (HTS) method.

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Background

- In order to identify novel drugs, and specifically leads for novel drugs, assays wherein large amounts of compounds are tested simultaneously for their biological properties are used extensively these days. The most commonly used format is
- 15 the binding assays, wherein samples are tested for their respective capabilities to displace a labelled known ligand from a receptor under investigation. However, this assay has several major drawbacks. The most important drawback of such competition assays is the fact that these assays are not functional assays, and therefore do not allow for any differentiation between agonists and antagonists.
- 20 This is of great importance when it comes to the study of receptors, the signalling of which is controlled in nature by agonists as well as by antagonists. The second major drawback of the competition assays is that since a receptor specific ligand must be identified, these assays can not be used for the investigation of orphan receptors, as no known ligand is known for these receptors.
- 25 Further, the labelling of the known ligand renders competition assays relatively cumbersome and therefore time consuming. Yet another disadvantage with conventional binding assays is that only compounds with affinity for the same binding site as the known ligand will be discovered and accordingly unknown binding sites on the receptor will not be discovered.

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Thus, in order to gain a higher degree of functional knowledge receptors expressed in whole living cells are often studied. Cells are then studied using a

focusing microscope, which method however requires much time and precision. A previously labelled sample is added to the microscope slide and an exact adjustment thereof is then required in order to obtain an informative picture. Since the conformation of a living cell is constantly changing with time, a certain skill is required with these methods. Reliable information regarding a receptor's structure, components and in certain cases its interaction with one specific ligand can thereby be obtained. However, whole cell studies using microscope are very time consuming and not convenient when the purpose is to study a large amount of candidate compounds' interactions with a receptor.

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US 5 891 646 in the name of Barak et al teaches a method for assaying receptor activity. More specifically, the interaction of a G-protein coupled receptor (GPCR) with β -arrestin conjugated to a detectable molecule is studied within cells and the β -arrestin redistribution from the cytosol to the plasma membrane is disclosed.

15 The purpose of the methods described therein is primarily an understanding of the mechanisms of action of various therapeutic agents. Furthermore, US 5 891 646 also proposes a method for screening for GPCR agonists and antagonists. However, such screening is also based on the use of whole living cells. This is a serious limitation in the screening of large libraries, since whole cells require a great degree of care when deposited on supporting substrates. Accordingly, these methods cannot be efficiently automated and are therefore not applicable in the context of the fast and simple high throughput screening methods, which are the most advantageous when compound libraries are screened.

25 Accordingly, there is a need within this field of novel methods that enable rapid and convenient screening of large numbers of candidate compounds for their biological effect on G-protein coupled receptors (GPCRs).

Summary of the invention

30 One object of the present invention is to provide a method for identifying biologically active compounds with a reduced degree of disturbance compared to the conventional binding assays discussed above. Another object of the invention

is to provide a method for identifying a biologically active compound, which method enables differentiating between agonists and antagonists. A further object of the invention is to provide a method which is suitable for use in high throughput screening. Accordingly, the method according to the present invention provides
5 the identification of novel drugs and/or drug leads in a faster and more convenient way than the above discussed assays.

More specifically, the present method provides the identification of a compound, which is capable of initiating the signalling of a G-protein coupled receptor
10 (GPCR). This is achieved by incubating a test compound with cell membranes from a GPCR expressing cell line, a kinase and a labelled arrestin, preferably a β -arrestin. This will allow the β -arrestin to bind to the GPCR, if the test compound is an agonist and causes the GPCR to be activated and phosphorylated. Then the mixture so obtained is contacted with carrier material capable of binding said cell
15 membranes, and signals emitted from the formed β -arrestin-GPCR-carrier complex are detected. Signals emitted from the carrier material are detected by any method, such as detection of light emitted, detection of radioactivity, e.g. scintillation proximity assay (SPA) beads (COOK, N. D., *Drug Discovery Today*, 1 (7), pp. 287-294, 1996: Scintillation proximity assay - a versatile high throughput
20 screening technology) etc. Such signals are then an indication of presence of an agonist in the sample. Other embodiments and aspects are as defined by the appended claims, and more details will be given below.

Detailed description of the invention

- 25 In a first aspect, the present invention relates to a method of identifying a compound capable of initiating the signalling of a G-protein coupled receptor (GPCR), which method comprises
- (a) contacting at least one test compound with cell membrane from at least one GPCR expressing cell or cell line, at least one kinase and at least one labelled
30 β -arrestin in a suitable buffer;
 - (b) allowing said β -arrestin to bind to activated and phosphorylated GPCR;

(c) contacting the resulting mixture with carrier material capable of binding said cell membrane(s); and

(d) detecting signals emitted from the formed β -arrestin-GPCR-carrier complex;

whereby a compound which is an agonist of the GPCR used is identified when

5 signals are emitted. In the present context, the separation of a desired signal from disturbing background noise may e.g. be performed by first measuring the signal emitted of the components without the test compound in order to establish a value of the background. Signals are then detected in step (d), and from that detection the background value is subtracted. The method used for the detection may e.g.
10 depend on the carrier material-label used. Below, the detection will be discussed in more detail.

Thus, as appears from the above, contrary to conventional binding assays, the present method can advantageously be used with orphan receptors, and it is also
15 easily adapted to an automated procedure due to the technical simplicity thereof.

G-protein coupled receptors (GPCRs) represent a large superfamily of proteins that transduce extracellular signals to the interior of cells, wherein each individual GPCR type activates a particular signal transduction pathway. Several different
20 signal transduction pathways are hitherto known to be activated via GPCRs. For example, the β 2-adrenergic receptors, which is a prototype mammalian GPCR, Dopamine D1a receptor or any other GPCR may be used in the present method. Signalling through GPCRs rapidly desensitizes, primarily as the consequence of receptor phosphorylation, even though receptor sequestration and downregulation
25 may also contribute to this process. Two families of serine/threonine kinases, second messenger dependent protein kinases and receptor-specific G-protein coupled receptor kinases, phosphorylate GPCRs and thereby contribute to receptor desensitisation. Receptor-specific phosphorylation of GPCRs promotes the binding of cytosolic proteins referred to as arrestins, which function to further
30 uncouple GPCRs from the heterotrimeric G-proteins. Both the kinase and the arrestin used in the present method will be discussed in more detail below.

Cell membranes from GPCR expressing cells or cell lines are prepared from cells capable of expressing the GPCR under investigation. More specifically, the present cell membranes may be from eukaryotic or prokaryotic cells, such as bacterial cells, yeast cells, fungal cells, insect cells, nematode cells, plant or
5 animal cells. Examples of animal cells are mammalian cells, such as HEK cells, HeLa cells, COS cells, CHO cells, BHK cells and various primary mammalian cells. Depending on the purpose of the investigation the cell, such as a cultured cell line or a cell isolated from a human being, is desintegrated into fragments of suitable sizes by standard methods, such as French press, sonication, physical
10 homogenisation etc.

Arrestin and β -arrestin are well known proteins which occur in nature in several forms, all of which are contemplated in the present context. The function of arrestin in nature is to deactivate a GPCR by binding to its activated and
15 phosphorylated form. Such binding causes the receptor signalling to stop and/or the receptor to be internalized. Thus, the arrestin used in the present method may e.g. be selected from the group consisting of β -arrestin 1, β -arrestin 2, β -arrestin 3 and β -arrestin 4. (For a general reference to arrestins, see e.g. Gurevich, JBC, vol 270, pp 720-731, 1995.)
20

The present test compounds are preferably obtained from libraries and may be peptides or other organic molecules. The test compounds may be organized according to standard methods into systems or arrays enabling a systematic testing of possible variations of chemical compositions. Chemical libraries, such as
25 combinatorial chemical libraries, comprise chemical compounds that have been synthesized from a systematic series of reactions. Such libraries can include an extraordinarily large and varied collection of compounds.

In the present method, the kinase may be a G-protein coupled receptor kinase
30 (GRK), a second messenger dependent protein kinase, or any other kinase capable of phosphorylating the GPCR. To date, the GRK protein family consists of six members, which can be further classified into subgroups according to

sequence homology and functional similarities, and any one of these, such as GRK-1 or GRK-2, may be used in the present context. (For a review of GRKs, see Palczewski, Eur. J. Biochem. vol 248, pp. 261-269, 1997). The person skilled in this field will realise that various combinations of GPCR-kinase may be tested in order to determine an optimal enzyme for the receptor used.

In an advantageous embodiment, the method described above is modified by the use of a mutant of β -arrestin, wherein the mutation renders the β -arrestin phosphorylation independent. Thereby the addition of kinase, such as G-protein coupled receptor kinase (GRK), can be excluded. Thus, such a method will comprise the following steps:

- (a) contacting at least one test compound with cell membrane from at least one GPCR expressing cell or cell line and at least one phosphorylation independent and labelled β -arrestin mutant in a suitable buffer;
- (b) allowing said β -arrestin mutant to bind to activated GPCR;
- (c) contacting the resulting mixture with carrier material capable of binding said cell membrane(s); and
- (d) detecting signals emitted from the formed β -arrestin-GPCR-carrier complex; whereby a compound which is an agonist of the GPCR used is identified when signals are emitted. As mentioned above, the skilled person will be able to select a suitable methodology to separate the signal emitted from formed β -arrestin-GPCR-carrier complex as an indication of bound β -arrestin from any disturbing background noise.

The mutant of β -arrestin may e.g. be selected from R169E (where amino acid number 169, arginine, is changed to glutamic acid) or 1-382 (where the β -arrestin has been truncated and only contains the first 382 amino acids) (see e.g. Krupnic, annu. rev. pharmacol. toxicol, vol 38, pp 289-319, 1998; and Koor, JBC, vol 274, pp 6831-6834, 1999). However, as the person skilled in this field will realize, novel useful phosphorylation independent mutants of β -arrestin may be created, the use of which are also within the scope of the present invention. In addition, the present invention also encompasses the use of any otherwise modified β -arrestin,

e.g. a protein from which one or more amino acids have been removed, i.e. subunits of β -arrestin, as long as it is capable of exerting its function of binding to activated GPCR in the present method.

5 The carrier material provides separation of GPCR bound β -arrestin from unbound β -arrestin by binding the GPCR- β -arrestin complex formed during the incubation step and may be any kind of support well known in this field. The carrier material can be omitted and separation of GPCR bound β -arrestin from unbound β -arrestin can be performed by other methods well known in the field, such as centrifugation,
10 precipitation and filtration, the use of such methods is also included in this patent. In an advantageous embodiment, the carrier material is in the form of beads, which are covered by a suitable binding partner to membranes capable of containing a GPCR, such as wheat germ agglutinate (WGA), antibodies against the receptor or antibodies against one or more inserted recognition sites in the
15 receptor. The preparation of an antibody raised against a known protein or peptide sequence is easily performed by the person skilled in this field. In a specifically advantageous embodiment of the present method, the carrier material also provides the signal which is detected in the last step, which signal is emitted when the label of the β -arrestin is sufficiently close to the carrier for an interaction
20 between the two. Thus, the detection step of the present method is uncomplicated, fast and efficient, since steps for separating bound β -arrestin from unbound β -arrestin are avoided. The label of the β -arrestin is advantageously a radioactive label. (For a reference to radio labelling and fluorescent labelling, see e.g. Atlas et al., Proc. Natl. Acad. Sci. USA 74:5490 (1977) and US patent number 5 576 436.)

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In a specifically advantageous embodiment, the present carrier material is scintillation proximity assay (SPA) beads where light is emitted when the beads recognize the presence of radioactively labelled β -arrestin bound to the receptor, where the receptor in turn has been bound to the beads via a layer of WGA. In the
30 most advantageous embodiment, the present method is a high throughput screening (HTS) assay, enabling the testing of a large number of samples in a short time. For a reference to such methods, see e.g. Picardo, M., Hughes, K.T.,

High Throughput Screening. The discovery of Bioactive Substances, pp. 307-316, Ed Devlin, J.P., 1997.

The use of the scintillation proximity assay (SPA) to study enzyme reactions and
5 receptor-ligand-interactions is a relatively new type of assay which however is well
known in the art by now. The great advantage of SPA technology over more
conventional assays or ligand-binding assays is that it eliminates the need to
separate unbound ligand from bound ligand prior to ligand measurement. (See for
example COOK, N. D., *Drug Discovery Today*, **1** (7), pp. 287-294, 1996:
10 Scintillation proximity assay - a versatile high throughput screening technology.)

The above described method is useful for screening large numbers of test
compounds in order to identify agonists for a G-protein coupled receptor, which
agonists are possible drugs. Such screening may e.g. be performed in microplates
15 using techniques and equipment well known to those of skill in screening
technology. Buffers for this purpose are also well known in the field, as illustrated
below in the section "Experimental". Drugs may be identified which are useful in a
vast number of clinical conditions, including e.g. diabetes, metabolic diseases, and
problems associated with vasodilation, cardiac disorders, bronchodilation, cancer
20 and endocrine secretion (see e.g. Lefkowitz et al., *Ann. Rev. Biochem.* 52:159
(1983)).

In a second aspect, the present invention relates to a method, wherein instead of
an agonist, an antagonist is identified. Thus, such a method of identifying a
25 compound capable of deactivating a G-protein coupled receptor (GPCR)
comprises

- (a) contacting cell membrane from at least one GPCR expressing cell or cell line
with at least one GPCR agonist, at least one kinase and at least one labelled
 β -arrestin in a suitable buffer;
- 30 (b) allowing said β -arrestin to bind to GPCR which has been activated by the
agonist and phosphorylated by the kinase;

(c) contacting at least one test compound with the resulting mixture to allow said test compound to bind to the GPCR and thereby displace any agonist previously bound thereto;

(d) contacting the mixture resulting from (c) with carrier material capable of binding
5 said cell membrane(s); and

(e) detecting signals emitted from the formed β -arrestin-GPCR-carrier complex;

whereby a compound which is an antagonist of the GPCR used is identified when a reduction in signal is observed. By "reduction" is meant a lower signal than the signal that may be detected after step (b), but before the addition of test
10 compound. Thus, the amount of GPCR bound β -arrestin is reduced after step (c) as compared to the amount bound after step (b). In this context, it is noted that all the methods according to the present invention may include use of a control and/or control measurements, as discussed above.

15 Agonists may have been obtained by the method described above or are known ligands for the studied receptors, e.g. hormones, amino acids, peptides, proteins or photons.

The details given above regarding the method for identification of agonists are also applicable in this aspect.

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In an advantageous embodiment, the above described method of identifying an antagonist can also be modified in order to exclude the use of phosphorylating enzyme, which is achieved by the use of a mutated β -arrestin. Thus, such a method comprises

25 (a) contacting cell membrane from at least one GPCR expressing cell or cell line with at least one GPCR agonist and at least one phosphorylation independent and labelled β -arrestin mutant in a suitable buffer;

(b) allowing said β -arrestin mutant to bind to GPCR which has been activated by said agonist;

30 (c) contacting at least one test compound with the resulting mixture to allow said test compound to bind to the GPCR and thereby displace any agonist previously bound thereto;

(d) contacting the mixture resulting from (c) with carrier material capable of binding said cell membrane(s); and

(e) detecting signals emitted from the formed β -arrestin-GPCR-carrier complex;

whereby a compound which is an antagonist of the GPCR used is identified when

5 a reduction in signal is observed.

The further details regarding this embodiment are as discussed above. Antagonists identified according to the present invention are e.g. useful as drugs, or as leads to design drugs, for the treatment and/or prevention of clinical
10 conditions, such as diabetes, metabolic diseases, cardiac disorders and cancer, and problems associated with vasodilation, bronchodilation and endocrine secretion (see e.g. Lefkowitz et al., Ann. Rev. Biochem. 52:159 (1983)).

In a third aspect, the present invention relates to the use of a compound identified
15 according to any one of the methods according to the invention as a lead compound, wherein the structure and/or biological properties of said compound are modified in order to provide a therapeutically effective substance. Such modification may e.g. be performed in order to eliminate or reduce undesired side effects in a human, enhance the biological properties thereof, facilitate the
20 preparation thereof, enhance the specificity of the action etc.

Thus, in a last aspect, the present invention relates to a method for producing a pharmaceutical preparation comprising a method as described above, which furthermore comprises the steps of mixing the compound identified with a
25 pharmaceutically acceptable carrier or transporter. Suitable transporters are well known in this field. The dosage and administration form of the final product will depend on the condition to be treated as well as on the age, sex, weight etc of the patient to be treated.

30 Experimental

The present examples are provided for illustrative purposes only and shall not be construed as limiting the scope of the present invention as defined by the

appended claims. All references given below and elsewhere in the present specification are hereby included herein by reference.

The assays described in the examples below are performed in 96 or 384 well microplates. Alternatively, other platforms such as 1536 well micro plates can be used, provided the added volumes and concentrations are corrected accordingly. All components are dissolved or suspended in assay buffer as follows: 50 mM Tris-HCl, pH 7.5, 50 mM potassium acetate, 0.5 mM $MgCl_2$, 1mM DTT, and 0.5mM ATP (ATP is not necessary when phosphorylation independent β -arrestin is used).

Example 1: Identification of agonists

To each well of a 96 well plate is added:

- 5 μ l sample which comprises a test compound is added to each well at 0.6 mg/ml. The test compound has been dissolved in DMSO and originates from a compound library.
- 50 μ l Dopamin D1a receptor containing cell membranes at 0.1 mg membrane/ml. The cell membranes are prepared from BHK cells expressing the receptor. Cell membranes are prepared using standard methods
- 50 μ l bovine GRK-2 at 10 μ M.
- 50 μ l radio labelled, bovine β -arrestin at 100000cmp/10 μ l, ^{125}I labelled β -arrestin is used.
- 50 μ l wheat germ agglutinate (WGA) coated scintillation proximity assay (SPA) (Amersham Pharmacia Biotech, Cardiff, Wales) beads at 15 mg/ml.

The resulting mixture is then incubated for one hour at room temperature with shaking, during which time complexes are allowed to form between activated and phosphorylated receptors and β -arrestin.

In order to detect agonists of the Dopamin D1a receptor, the plates are read/counted in a TopCount (from Packard Instrument Company). Light is

detected as an indication of the presence of agonist, since labelled β -arrestin binds to activated and phosphorylated receptor, i.e, to receptor activated by a test compound capable of acting as an agonist. The receptor is in turn bound to SPA beads, thus enabling light emission as a result of the interaction bead-radioactive label.

Accordingly, test compounds present in the samples added to the wells from which light is detected are selected for further investigation as possible drugs.

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Example 2: Identification of agonists using a β -arrestin mutant

To each well of a 384 well plate is added:

-1 μ l sample which comprises a test compound is added at 0.6 mg/ml. The test compound is from a compound library and has been dissolved in water.

-10 μ l GPCR (G-protein coupled receptor) containing cell membranes at 0.1 mg membrane/ml. Cell membranes are prepared from cells expressing the GPCR. Cell membranes are acquired from Euroscreen (www.euroscreen.be/UGARF.htm).

-10 μ l radio labelled β -arrestin R169E (wherein amino acid no. 169, arginine, has been changed to a glutamic acid), 100000 cpm/10 μ l, 125 I labelled β -arrestin is used.

10 μ l wheat germ agglutinate (WGA) coated Leadseeker beads (from Amersham Pharmacia Biotech, APB) at 15 mg/ml.

25

The resulting mixture is then incubated for one hour at room temperature, during which time complexes are allowed to form between activated receptors and phosphorylation independent β -arrestin.

30 The plate is counted/read using a Leadseeker CCD camera (from Amersham Pharmacia Biotech, APB). As in Ex 1 above, light emission is an indication of an

agonist. Accordingly, test compounds added to the wells from which light is emitted are selected for further investigation as possible drugs.

5 Example 3: Identification of an antagonist

To each well of a 384 well plate is added:

-10 μ l agonist in a concentration sufficient to provide activation of essentially all of the receptors present in each well.

- 10 -1 μ l sample which comprises a test compound is added at 0.6 mg/ml. The test compound is from a compound library and has been dissolved in water.

-10 μ l GPCR (G-protein coupled receptor) containing cell membranes at 0.1 mg membrane/ml. Cell membranes are prepared from cells expressing the GPCR under investigation.

- 15 -10 μ l bovine GRK-2 at 10 μ M.

-10 μ l radio labelled β -arrestin, 100000 cpm/10 μ l, 125 I labelled β -arrestin is used.

10 μ l wheat germ agglutinate (WGA) coated Leadseeker beads (from Amersham Pharmacia Biotech, APB) at 15 mg/ml.

- 20 The resulting mixture is then incubated for one hour at room temperature, during which time complexes are allowed to form between activated and phosphorylated receptors and β -arrestin.

The plate is counted/read in a Leadseeker as described above. A reduction of light
25 emission is here an indication of a test compound capable of acting as an antagonist. Thus, test compounds from which such a reduction is detected are selected for further investigation as drugs.

30 Example 4: Identification of an antagonist using a β -arrestin mutant

To each well of a 384 well plate is added:

-10µl agonist in a concentration sufficient to activate a detectable amount of the receptors present in each well.

-1µl sample which comprises a test compound is added at 0.6 mg/ml. The test compound originates from a compound library and has been dissolved in DMSO.

5 -10µl GPCR (G-protein coupled receptor) containing cell membranes at 0.1 mg membrane/ml. Cell membranes are prepared from cells expressing the GPCR under investigation. The cell membranes are prepared using standard methods.

-10µl radio labelled β -arrestin R169E, 100000cmp/10µl, 125 I labelled β -arrestin is used.

10 10µl wheat germ agglutinate (WGA) coated Leadseeker beads at 15mg/ml.

The resulting mixture is then incubated for one hour at room temperature, during which time complexes are allowed to form between activated receptors and phosphorylation independent β -arrestin.

15

The plate is counted/read in a Leadseeker as described above. As in Ex 3, a reduction in light emission is an indication of a test compound capable of acting as an antagonist. Thus, test compounds from which such a reduction is observed are selected for use as lead compounds in the development of drugs.

20

CLAIMS

1. A method of identifying a compound capable of initiating the signalling of a G-protein coupled receptor (GPCR), which method comprises
 - 5 (a) contacting at least one test compound with cell membrane from at least one GPCR expressing cell or cell line, at least one kinase and at least one labelled β -arrestin in a suitable buffer;
 - (b) allowing said β -arrestin to bind to activated and phosphorylated GPCR;
 - (c) contacting the resulting mixture with carrier material capable of binding said
10 cell membrane(s); and
 - (d) detecting signals emitted from the formed β -arrestin-GPCR-carrier complex;whereby a compound which is an agonist of the GPCR used is identified when signals are emitted.
2. A method according to claim 1, wherein the kinase is G-protein coupled
15 receptor kinase (GRK), such as GRK-2.
3. A method of identifying a compound capable of initiating the signalling of a G-protein coupled receptor (GPCR), which method comprises
 - (a) contacting at least one test compound with cell membrane from at least one
20 GPCR expressing cell or cell line and at least one phosphorylation independent and labelled β -arrestin mutant in a suitable buffer;
 - (b) allowing said β -arrestin mutant to bind to activated GPCR;
 - (c) contacting the resulting mixture with carrier material capable of binding said cell membrane(s); and
 - (d) detecting signals emitted from the formed β -arrestin-GPCR-carrier complex;
25 whereby a compound which is an agonist of the GPCR used is identified when signals are emitted.
4. A method according to claim 3, wherein said β -arrestin mutant is R169E- β -arrestin or 1-382- β -arrestin.
5. A method of identifying a compound capable of deactivating a G-protein
30 coupled receptor (GPCR), which method comprises

- (a) contacting cell membrane from at least one GPCR expressing cell or cell line with at least one GPCR agonist, at least one kinase and at least one labelled β -arrestin in a suitable buffer;
 - (b) allowing said β -arrestin to bind to GPCR which has been activated by the agonist and phosphorylated by the kinase;
 - (c) contacting at least one test compound with the resulting mixture to allow said test compound to bind to the GPCR and thereby displace any agonist previously bound thereto;
 - (d) contacting the mixture resulting from (c) with carrier material capable of binding said cell membrane(s); and
 - (e) detecting signals emitted from the formed β -arrestin-GPCR-carrier complex; whereby a compound which is an antagonist of the GPCR used is identified when a reduction in signal is observed.
6. A method according to claim 5, wherein the kinase is G-protein coupled receptor kinase (GRK), such as GRK-2.
7. A method of identifying a compound capable of deactivating a G-protein coupled receptor (GPCR), which method comprises
- (a) contacting cell membrane from at least one GPCR expressing cell or cell line with at least one GPCR agonist and at least one phosphorylation independent and labelled β -arrestin mutant in a suitable buffer;
 - (b) allowing said β -arrestin mutant to bind to GPCR which has been activated by said agonist;
 - (c) contacting at least one test compound with the resulting mixture to allow said test compound to bind to the GPCR and thereby displace any agonist previously bound thereto;
 - (d) contacting the mixture resulting from (c) with carrier material capable of binding said cell membrane(s); and
 - (e) detecting signals emitted from the formed β -arrestin-GPCR-carrier complex; whereby a compound which is an antagonist of the GPCR used is identified when a reduction in signal is observed.
8. A method according to claim 7, wherein said β -arrestin mutant is R169E- β -arrestin or 1-382- β -arrestin.

9. A method according to any one of the preceding claims, wherein the signal emitted by the carrier material is light due to scintillation and the β -arrestin is radioactively labelled.
- 10.A method according to claim 9, wherein the carrier material comprises
5 scintillation proximity assay (SPA) beads.
- 11.A method according to any one of the preceding claims, wherein the carrier material is provided with wheat germ agglutinate (WGA) to allow binding of cell membrane(s) expressing GPCR(s).
- 12.A method according to any one of the preceding claims, which is a high
10 throughput screening method.
- 13.Use of a compound identified according to the method defined in any one of the preceding claims as a lead compound in drug design, wherein structure and/or biological properties of said compound are modified in order to provide a therapeutically effective substance.
- 15 14.A method for producing a pharmaceutical preparation comprising a method according to any one of claims 1-12, furthermore comprising the steps of mixing the compound identified with a pharmaceutically acceptable carrier.

ABSTRACT

The present invention relates to a method for identifying compounds capable of affecting the activity of G-protein coupled receptors (GPCRs), i.e. identifying
5 agonists or antagonists of said receptor type. Such compounds are later useful e.g. as drugs or as leads in drug development. More specifically, if agonists are desired, the method comprises contacting a test compound with cell membranes from a GPCR expressing cell line, a kinase, such as G-protein coupled receptor kinase (GRK), and labelled β -arrestin. If the test compound is an agonist it will
10 allow said β -arrestin to bind to activated and phosphorylated GPCR. The above mentioned mixture is then contacted with carrier material capable of binding GPCRs, and signals are detected as an indication that the test compound is capable of acting as an agonist of a GPCR.

If on the other hand receptor antagonists are desired, the present method is
15 modified to be a competition assay, wherein known agonists are also added to the incubation mixture and compounds are tested as to their capability to displace agonists bound to the receptor, and thereby dissociate labelled β -arrestin from the receptor. In both of the above described methods, the kinase and β -arrestin may be replaced by a phosphorylation independent mutant of β -arrestin, omitting the
20 use of a kinase.